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PROTOCOL FOR

THE ASSESSMENT OF IMMUNE MECHANISMS INFLUENCED BY VINYL CHLORIDE EXPOSURE IN RABBITS AND MICE

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INTRODUCTION

Vinyl chloride causes hepatic fibrosis that may lead to portal hypertension and angiosarcoma in liver. The so-called vinyl chloride syndrome is sometimes associated with dermal induration and acro-osteolysis (Harris and Adams, 1967), splenomegaly and thrombocytopenia (Lange et al., 1974). Similar effects involving miscellaneous types of tumors have been reported in animals (Maltoni and LeFemine, 1974).

In a recent report, Ward et al. (1976) suggested the presence of immune complexes in workers exposed to vinyl chloride. In most cases, these complexes were associated with clinical syndrome. An increase in immunoglobulins and a reduction in peripheral T-lymphocytes was reported (although none of these appeared to be of statistical significance). These workers suggested that the vinyl chloride disease is an immune complex disorder and the immune response is initiated by the adsorption of vinyl chloride or a metabolite on the tissue or plasma proteins.

In another report, Ott et al. (1974) suggested the immune suppression in vinyl chloride exposed workers on the basis of the occurrence of miscellaneous types of tumors not necessarily related to hemangiosarcoma. A research proposal from Mario Negri Institute (1974) also suggested the need of evaluation of immunosuppressive role of vinyl chloride.

Lange et al. (1974) found both increases and decreases of different fractions of immunoglobulins in 10 patients suffering from vinyl chloride

disease and on this basis excluded the possibility of autoimmune phenomenon in this syndrome.

Based on these reports it appears that a controlled study in laboratory animals is needed to provide information either supporting or excluding the autoimmune phenomenon or immune suppression in vinyl chloride exposure. Since immune suppression can be produced by various antigens or antibodies (Rowley, et al. 1973) these two are not necessarily mutually exclusive.

OBJECTIVE

The objective of this study is to investigate the possible role of immune alterations in vinyl chloride syndrome, either through autoimmune complexes or immune suppression, or both.

EXPERIMENTAL

Animal Species: The two species of animals used will be rabbit and mouse. White New Zealand male rabbits (2-3 kg body weight) will be obtained from Langshaw Rabbitory, Carson City, MI. Male CD-1 mice, approximately 8 weeks old will be obtained from Charles River. The number of animals per group is indicated later. All animals will be observed daily and weighed weekly.

Housing and Exposure: The rabbits will be housed individually and allowed free access to feed and water except during exposure. The mice will be kept in groups. The cages of all animals will be kept in large inhalation chambers and will be left there throughout the duration of the experiment.

Vinyl chloride (obtained from Matheson Gas Products) will be used. Exposures will be 0, 10, 100 and 1000 ppm of vinyl chloride in air 6 hours per day, 5 days per week for 8 weeks. The concentration of vinyl chloride in inhalation chambers will be monitored by gas chromatography.

Schedule for rabbits: Five rabbits will be used for each exposure level. Blood from all rabbits will be collected by puncturing the ear vein at pre-exposure, and 4, 6 and 8 weeks after exposure begins. The blood will be used for RBC, WBC and differential leukocyte counts and serum obtained for electrophoresis of immunoglobulins. After 4 weeks of exposure, these animals will be injected with a mixture of Freund's complete adjuvant and tetanus toxoid. The antibody titers will be determined in subsequent serum samples by hemagglutination procedure. Antigens will be repeated 2 weeks later.

Approximately 10 and 24 days after the antigen challenge the animals will be tested for skin sensitivity to tuberculin by intradermal injection. The response will be measured 24 and 48 hours after the injections.

At the end of the 8 week exposure period, the animals will be sacrificed by decapitation and their liver, kidney, spleen, thymus, adrenals, and

popliteal lymph nodes will be collected and weighed. The organ/body weight ratios will be calculated. Parts of liver, kidney, lymph node, spleen and thymus will be preserved in 10% buffered formalin for histopathologic examination. One lymph node will be frozen for subsequent evaluation of plasma cells using immunofluorescent techniques. Part of the spleen will be used for splenic lymphocyte cultures in vitro to evaluate the sensitivity of lymphocytes to various mitogens (see lymphocyte cultures).

Schedule for mice: Mice will be sacrificed at pre-exposure and 2,4 and 8 week exposure periods. The groups will be arranged to allow the sacrifice of at least 4 animals from each group at each time interval. An additional group of 4 animals at each exposure level will be injected with the antigen (tetanus toxoid) after 4 and 6 weeks of exposure and the antibody titers determined at the end of the exposure period.

All animals will be sacrificed by decapitation and the blood collected. Total RBC, WBC and differential leukocyte counts will be made. Serum will be collected for immunoelectrophoresis, and antibody titers in suitable samples. Liver, kidneys, thymus and spleen will be weighed and their organ/body weight ratios determined. The spleen will be saved in sterilized isotonic saline solution for lymphocyte cultures in vitro. Liver, kidney and thymus will be fixed in 10% buffered formalin for histopathologic examination.

Histopathologic evaluation: All tissues preserved for histopathologic examination will be routinely processed and stained by H. and E. These will be examined under a light microscope.

Fluorescence visualization of plasma cells in lymph nodes of rabbits: The popliteal lymph nodes will be frozen, sectioned in a cryostat and incubated with fluorescein-conjugated anti-rabbit globulin. After gentle washing, the sections will be examined under a microscope in ultraviolet light and the number of fluorescent cells will be counted. The processing and evaluation of lymph nodes will be performed in a random blind fashion.

Splenic lymphocyte cultures: Lymphocytes will be isolated from spleens as soon as possible after collecting the organs and suspended in a suitable media. Several replicates of these will be incubated in system containing no mitogen, phytohemagglutinin or pokeweed mitogen. After 52 hours of culturing, ^{3}H-thymidine will be added to cultures. Nearly 16 hours after this addition, the cells will be harvested, washed thoroughly in saline and counted by liquid scintillation to determine the incorporation of labelled thymidine into nuclear DNA. The blastogen induced DNA synthesis in relation to the cultures containing no mitogen (stimulation index) will be compared in groups with different levels of exposure of vinyl chloride.

Statistical: The results will be compared by variance ratio test. The level of $p<0.05$ will be used for significance.

COST OF THE EXPERIMENT

The total cost of this project is anticipated to be $45,000.

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